

What Is Claimed Is:

1. A method of making a glycosylated antibody having a reactive ketone group on the glycosylated site, comprising:

expressing a cell transfected with a vector encoding an antibody having one or more glycosylation sites in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor.

2. The method of claim 1, wherein the ketone derivative of the saccharide or saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

3. The method of claim 1, wherein the antibody has a glycosylation site in a domain selected from the group consisting of the V κ domain and the CH1 domain.

4. The method of claim 1, wherein the antibody has more than one glycosylation site.

5. The method of claim 1, wherein the antibody is a single-chain antibody.

6. A method of making a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, comprising:

expressing a cell transfected with a vector containing an antibody having one or more glycosylation sites in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor, and

fragmenting the resulting antibody into an antigen-binding antibody fragment.

7. The method of claim 6, wherein the fragment is an F(ab')₂ fragment.

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8. A method of making an immunoconjugate comprising a glycosylated antibody conjugated to an agent through its glycosylated site, comprising:

expressing a cell transfected with a vector containing an antibody having one or more glycosylation sites in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,

reacting the resulting antibody with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides.

9. The method of claim 8, wherein the antibody is purified before reaction with the agent.

10. The method of claim 8, wherein the agent is added directly to the culture medium.

11. The method of claim 8, wherein the antibody is immobilized on a protein A column prior to reaction with the agent, and eluted from the protein A column after reaction with the agent.

12. The method of claim 8, wherein the agent is selected from the group consisting of diagnostic agents, therapeutic agents, chelating agents and linking agents.

13. The method of claim 12, wherein the agent is selected from the group consisting of peptides, oligosaccharides, biotinamidocaproyl hydrazides, diagnostic markers, drugs, toxins, imaging radioisotopes, and therapeutic radioisotopes.

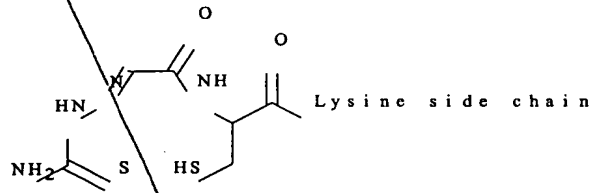
14. The method of claim 8, wherein the agent is a ligand-containing peptide selected from the group consisting of DTPA-bearing peptides, DOTA-bearing peptides, $\text{AcK}_d\text{D}_d\text{K}_d(\text{TscGC})\text{D}_d\text{K}_d\text{-NH}(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{CONH-NH}_2$, $\text{AcK}_d\text{D}_d\text{K}_d(\text{TsdGC})\text{D}_d\text{K}_d\text{-NH}(\text{CH}_2)_4\text{H}(\text{NH}_2)\text{CONH-NH}_2$, and $\text{H}_2\text{N-NH-CH}_2\text{-CO-}$

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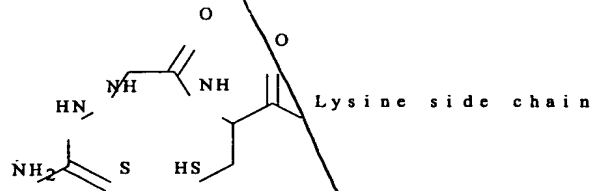
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$D_d-K_d(\text{TscGC})-D_d-K_d-\text{NH}_2$, where K_d and D_d represent the D-amino acids D-lysine and D-aspartic acid, respectively, and where TscGC is the ligand:



and TsdGC is the ligand:



15. The method of claim 14, wherein the agent is $\text{H}_2\text{N}-\text{NH}-\text{CH}_2-\text{CO}-D_d-K_d(\text{TscGC})-D_d-K_d-\text{NH}_2$.

16. A method of making an immunoconjugate comprising a glycosylated antigen-binding antibody fragment conjugated to an agent through the glycosylated site, comprising:

expressing a cell transfected with a vector containing an antibody having one or more glycosylation sites in a culture medium comprising a ketone derivative of a saccharide or saccharide precursor,

fragmenting the resulting antibody into an antigen-binding antibody fragment, and

reacting the antibody fragment with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides.

17. The method of claim 16, wherein the fragment is an $F(ab')_2$ fragment.

18. The method of claim 16, wherein the agent is selected from the group consisting of diagnostic agents, therapeutic agents, chelating agents and linking agents.

19. A glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site.

20. The glycosylated antibody or antigen-binding antibody fragment of claim 19, wherein the antibody or antibody fragment is glycosylated in a domain selected from the group consisting of the V_k domain and the CH1 domain.

21. The glycosylated antibody or antigen-binding antibody fragment of claim 19, wherein the antibody or antibody fragment has more than one glycosylation site, each of which has a reactive ketone group.

22. An immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an agent through the glycosylated site.

23. The immunoconjugate of claim 22, wherein the glycosylated site is in a domain selected from the group consisting of the V_k domain and the CH1 domain.

24. The immunoconjugate of claim 22, wherein the antibody has more than one glycosylated site, each of which is conjugated to an agent.

25. The immunoconjugate of claim 22, wherein the agent is selected from the group consisting of diagnostic agents, therapeutic agents, chelating agents and linking agents.

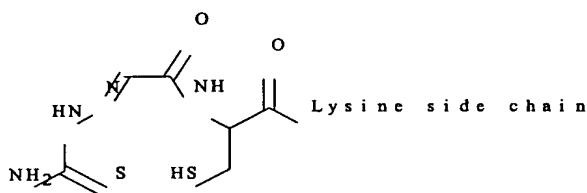
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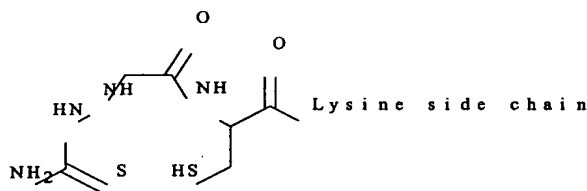
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26. The immunoconjugate of claim 25, wherein the agent is selected from the group consisting of peptides, oligosaccharides, biotinamidocaproyl hydrazides, diagnostic markers, drugs, toxins, imaging radioisotopes, and therapeutic radioisotopes.

27. The immunoconjugate of claim 25, wherein the agent is a ligand-containing peptide selected from the group consisting of DTPA-bearing peptides, DOTA-bearing peptides, $\text{AcK}_d\text{D}_d\text{K}_d(\text{TscGC})\text{D}_d\text{K}_d\text{-NH}(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{CONH-NH}_2$ and $\text{AcK}_d\text{D}_d\text{K}_d(\text{TsdGC})\text{D}_d\text{K}_d\text{-NH}(\text{CH}_2)_4\text{H}(\text{NH}_2)\text{CONH-NH}_2$, where K_d and D_d represent the D-amino acids D-lysine and D-aspartic acid, respectively, and where TscGC is the ligand:



and TsdGC is the ligand:



28. The immunoconjugate of claim 27, wherein the agent is $\text{H}_2\text{N-NH-CH}_2\text{-CO-D}_d\text{-K}_d\text{-(TscGC)-D}_d\text{-K}_d\text{-NH}_2$.

29. The immunoconjugate of claim 22, wherein the agent is a chelating agent chelated to a diagnostic or therapeutic radioisotope.

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30. A method of targeting an active agent to an *in vivo* target site comprising administering an immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an active agent through the glycosylated site.

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31. The method of claim 30, wherein the active agent is selected from the group consisting of diagnostic and therapeutic agents.

32. The method of claim 30, wherein the antibody or antibody fragment has multiple glycosylated sites, each of which is conjugated to an active agent.

33. A method of targeting an active agent to an *in vivo* target site comprising:

administering a glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylation site, and allowing the antibody or antibody fragment to localize at the target site;

optionally, administering a clearing agent to clear non-localized antibody or antibody fragment from circulation; and

administering an active agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, whereby the active agent localizes at the target site via conjugation with the pre-targeted antibody or antibody fragment.

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34. The method of claim 33, wherein the active agent is selected from the group consisting of diagnostic and therapeutic agents.

35. The method of claim 33, wherein the clearing agent is administered.

36. The method of claim 35, wherein the clearing agent is an anti-idiotypic clearing agent.

37. The method of claim 33, wherein the antibody or antibody fragment has more than one glycosylated site, and wherein more than one active agent moiety is conjugated to the pretargeted antibody or antibody fragment.

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